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**DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC
METHOD FOR ENZALUTAMIDE IN STANDARD AND DOSAGE FORM
BY FIRST DERIVATIVE SPECTROPHOTOMETRIC METHOD**

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ABSTRACT

A simple, rapid, accurate and economical First order UV-derivative spectrophotometric method has been developed for estimation of Enzalutamide from bulk and pharmaceutical formulation. The λ_{max} of Enzalutamide in methanol was found to be 236nm. The same spectrum was derivatized into first order derivative; showed maxima at 226 nm and minima at 258 nm. The drug follows linearity in the concentration range 05-15 μ g/ml with correlation coefficient value 0.9993. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 101.12% was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120%. The % recovery was found to be in the range 101.67%–102.40%. The low values of %R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The %R.S.D. value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of Enzalutamide in bulk and in pharmaceutical dosage form.

Keywords: Enzalutamide; Validation; first order derivative UV, quantitative determination, methanol

INTRODUCTION

Enzalutamide chemically is 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5-dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-N-methylbenzamide [5-6] prostate cancer is the typical cause of cancer-related death; Although most patients initially respond to androgen-deprivation therapy, prostate cancer eventually progresses to castration-resistant prostate cancer (CRPC). Effective treatment options for Metastatic castration-resistant prostate cancer are lacking, and the median survival for men with mCRPC is <2 years [1].

The androgen receptor (AR) is central to the biology of metastatic castration resistant prostate cancer. The mechanisms that explain resistance to traditional androgen deprivation therapy include AR amplification or point mutations, ligand-independent activation of the AR, and alternative signaling pathways that no longer involve the AR. Androgen antagonists, including Enzalutamide, have been shown to exhibit agonist activity when AR is overexpressed [2].

ENZ is a second generation androgen receptor inhibitor. It competitively inhibits androgen binding to androgen receptors and inhibits androgen receptor nuclear translocation and interaction with DNA. Enzalutamide is androgen receptor inhibitor

with clinically significant clinical activity in patients with metastatic castration-resistant prostate cancer following docetaxel based chemotherapy [3].

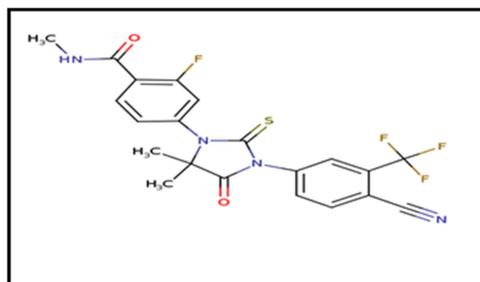


Figure 1: Structural formulas of ENZ

Literature survey revealed that Enzalutamide is not official in any pharmacopoeia but determined by various methods including Simple UV spectrophotometry and LC-MS-MS. Literature survey reveals that only LC-MS methods were reported for the determination of enzalutamide in Animal plasma & Brain homogenates and in human plasma [4].

whereas one uv method was reported. Among the various methods available for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost. This study presents new spectrophotometric method for the determination of Enzalutamide in bulk and pharmaceutical formulations. Accordingly, the objective of this study was to develop and validate the first order derivative method for the

estimation of Enzalutamide in bulk and pharmaceutical formulation as per ICH guidelines [5].

MATERIAL AND METHODS

Enzalutamide, Capsule formulation (Xtadi), Label claim: Enzalutamide - 40 mg, AR grade methanol (Finar Chemical Ltd., Ahmedabad, India), Electronic analytical balance – Acculab ALC 210.4, Ultra sonicator (EN 30 US, Energetech Fast Clean, Mumbai, India,) A double beam UV-Visible spectrophotometer (model UV-1800 of Shimadzu Corporation (Japan) with spectral bandwidth of 2 nm, wavelength accuracy of ± 0.5 nm and a pair of 10 mm matched quartz cells) [6].

Spectrophotometric Conditions

Mode: Spectrum, Scan speed: Fast, Wavelength range: 400-200 nm, Absorbance scale: 0.00A – 4.00A, Initial baseline correction: Methanol AR [7].

Preparation of standard solution:

Preparation of Standard Stock Solution of Enzalutamide (1000 $\mu\text{g}/\text{mL}$)

A standard stock solution containing 1000 $\mu\text{g}/\text{mL}$ of ENZ was prepared by Dissolving accurately weighed 50 mg quantity of ENZ in methanol and diluting to volume with methanol in a 50 mL volumetric flask. After that Pipette out 1 mL of Stock solution in 10 mL Volumetric flask to make 100 $\mu\text{g}/\text{mL}$ Solution [8].

Preparation of Standard Calibration Solutions of ENZ (5-15 $\mu\text{g}/\text{mL}$)

Standard calibration solutions having concentrations of 0.5, 0.7, 0.9, 1.0, 1.3 and 1.5 $\mu\text{g}/\text{mL}$ of ENZ were prepared in 10 mL volumetric flasks by taking appropriate aliquots from the standard stock solution of ENZ and diluting them to volume with methanol [9].

Test Sample Preparation

Twenty Xtadi Cap were accurately weighed, calculate average weight. An amount of Capsule equivalent to 40 mg ENZ was weighed and transferred into a 50 mL Volumetric flask and 30 mL of methanol was added into it. The contents of the flask were sonicated for 15 min to dissolve the active ingredients completely. The solution was then diluted to 40 mL with methanol and solution was filtered through whatman filter paper. Take 0.1 mL aliquot from the filtered solution was transferred into a 10 mL volumetric flask to get concentration 10 $\mu\text{g}/\text{mL}$ ENZ was then analysed for assay determination.

Derivative Condition

The standard drug solution was diluted so as to get the final concentration in the range of 5-15 $\mu\text{g}/\text{ml}$ and scanned in the first order derivative spectra. The first order derivative spectra at showed a maxima and minima at

226 and 258 nm respectively. The amplitude of absorbance was measured at 226 nm (peak maxima) and at 258 nm (peak minima) and Take Aptitude Difference was plotted against concentration to give calibration curve, and regression equation was calculated. The amplitude was linear in the concentration range of 5-15 µg/ml.

Method validation

As per ICH guidelines (Q2R1), the method validation parameters studied were specificity, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

Linearity

Linearity is expressed in terms of correlation co-efficient of linear regression analysis. From stock solution 5-15 µg/mL concentration range solutions are prepared in methanol solvent. Under the experimental conditions described, the graph obtained for first order derivative spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves was $y = 0.0029x - 0.002$ ($r^2 = 0.9993$) for first order derivative spectrophotometry. The range was found to be 5-15 µg/mL for first order derivative spectrophotometric methods. The calibration curves are showed in **Figures 1**. The

absorbance of each solution was measured at described under **Table 1**.

Accuracy (% Recovery)

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of ENZ to reanalyzed solutions of commercial Capsule. Measure the Absorbance of the standard solution, Accuracy 90%, Accuracy 100% and Accuracy 110% solutions, Calculate the amount found and amount added for ENZ, calculate the individual recovery and mean recovery values. The results are shown in **Table 2**.

Precision

Repeatability (Intra-day Precision): Repeatability (Intra-day precision) was determined by analyzing of ENZ standard solutions in the range 9, 10, 11 µg/mL for first order derivative spectrophotometric in triplicate. Calculate % RSD for ENZ.

Inter-day Precision: Inter-day precision was determined by analyzing of ENZ standard solutions in the range 9, 10 and 11 µg/mL for first order derivative spectrophotometric in triplicate on the different days. Calculate % RSD for ENZ.

- **Limit of Detection and Limit of Quantification:** The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the standard deviation of y-intercept of calibration curve (N) and slope (S) of the calibration curve.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ is the SD of the response

S is the slope of the calibration curve.

RESULTS AND DISCUSSION

The satisfactory results were obtained with methanol for better reproducibility and repeatability, as ENZ is soluble in methanol. The derivative graphical method was used to estimate ENZ. The overlaid first-derivative spectrum of ENZ was found to be appropriate for the determination ENZ by spectra showed at a maxima and minima in methanol. The spectrum of ENZ has zero order absorbance at 236 nm, where ENZ gives a significant derivative response, maxima at 226 nm and minima at 258 nm, which is near to the zero order absorbance.

Method Validation

Linearity

The Beer's law was obeyed. Linear correlation was obtained between absorbance and concentrations of ENZ in the range of

and 5-15 $\mu\text{g/mL}$, respectively. The linearity of the calibration curve was validated by the value of correlation coefficients of the regression (r^2).

Precision

The % RSD for repeatability (intra-day) precision was found to be in the range of 0.52-0.99%. while inter-day precision was found to be in the range of 0.91-1.66% for ENZ, which indicates the method is precise.

Accuracy (% Recovery)

The accuracy study was carried out by the standard addition method. The percent recovery was found in the range of 101.67-102.40% for ENZ and, which indicates accuracy of the method.

Limit of detection and limit of quantification

The Limit of detection (LOD) was found to be 0.23 $\mu\text{g/mL}$ and the Limit of quantification (LOQ) was found to be 0.72 $\mu\text{g/mL}$ and 0.38 $\mu\text{g/mL}$ for Enzalutamide.

Analysis of marketed formulation

The proposed derivative method was successfully applied for determination of ENZ from there marketed dosage form. The percentage of ENZ was found to be satisfactory, which is comparable with the corresponding label claim amount.

Table 1: Linearity data for Enzalutamide

Parameter	Result	
Absorption maxima and minima (nm)	226	258
Beer's-Lamberts range ($\mu\text{g/ml}$)	5 - 15	
Regression equation $y=mx+c$	$Y= 0.0029x-0.002$	
Slope(m)	0.0029	
Intercept(c)	0.002	
Correlation coefficient (r2)	0.9993	

Table 2: Results of Intra-day precision and Inter-day precision

Concentration ($\mu\text{g/mL}$)	Inter-day precision		Intra-day precision	
	Amount Found \pm S.D (n=3)	%RSD	Amount Found \pm S.D (n=3)	%RSD
9	0.026 \pm 0.000153	0.58	0.0261 \pm 0.000351	1.36
10	0.0291 \pm 0.000153	0.52	0.0293 \pm 0.000265	0.91
11	0.032 \pm 0.000321	0.99	0.0328 \pm 0.000551	1.66

Table 3: Recovery data for ENZ

Amount of sample ($\mu\text{g/mL}$)	Amount of Standard added ($\mu\text{g/mL}$)	Amount found Mean* \pm S.D ($\mu\text{g/mL}$)	%Recovery	%RSD
5	4	9.08	102.00	0.116
5	5	10.12	102.40	0.156
5	6	11.18	101.67	0.261

Table 4: LOD and LOQ for ENZ

ENZ ($\mu\text{g/mL}$)	
LOD	LOQ
0.23	0.72

Table 5: Analysis marketed formulation

Drug	Amount of drug (mg)		%Assay (n=6)	% RSD
	Labelled	Estimated		
Enzalutamide	40	40.4	101.12	1.117

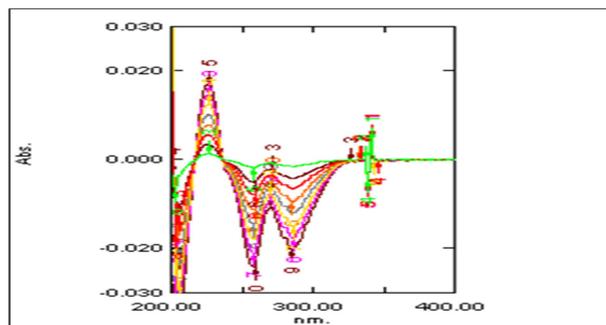


Figure 2: Linearity for first order spectra of ENZ

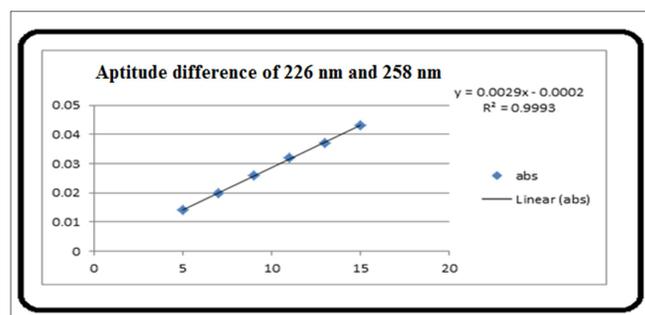


Figure 3: Calibration curve of ENZ

CONCLUSION

This first order UV spectrophotometric derivative technique is quite simple, accurate, precise, reproducible and sensitive. The first order UV derivative method has been developed for quantification of Enzalutamide in Capsule formulation. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound.

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